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Neonatal Behavioral Assessment Scale Performance in Humans Influenced by Maternal Consumption of Environmentally Contaminated Lake Ontario Fish

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ABSTRACT. Behavioral effects in neonates of their mothers' consumption of Lake Ontario fish were examined using the Neonatal Behavioral Assessment Scale (NBAS). Newborns of women who had consumed > 40 equivalent pounds of fish were placed in a high-exposure group (n = 152), those of women who had consumed < 40 equivalent pounds of fish were placed in a low-exposure group (n = 243), and offspring of women who reported having never eaten Lake Ontario fish comprised the control group (n = 164). Assessments were given at 12–24 hours after birth, and again at 25–48 hours after birth. Despite relatively low levels of fish consumption, newborns in the high-exposure group scored more poorly than those in both the low-exposure and control groups on the Reflex, Autonomic, and Habituation clusters of the NBAS. No significant group differences were found on the Orientation, Range of State, Regulation of State, or Motor clusters of the NBAS, nor did birth weight or head circumference differ between groups. These results represent the first replication and extension of the neonatal results of the Lake Michigan Maternal Infant Cohort study (Jacobson et al. 1984).

INDEX WORDS: Behavioral teratogens, Great Lakes fish, human development, Neonatal Behavioral Assessment Scale.

INTRODUCTION

The Oswego Newborn and Infant Development Project was begun to examine the behavioral effects in human newborns, infants, and children of maternal consumption of Lake Ontario fish that were contaminated with a wide range of persistent toxic chemicals such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), polychlorinated dibenzo-*p*-dioxins, dieldrin, lindane, chlordane, cadmium, mercury, and mirex. Located on the southeastern shore of Lake Ontario, Oswego County is a mecca for sport fishing. Data collected by Dawson and Brown (1989) and Vena (1992) indicated that the decade of the 1980s witnessed a dramatic increase in sport fishing in the area, with various surveys indicating upwards of 50% of anglers eating some or all of the fish they caught. New York State Department of Health has published advisories that women of childbearing age should not consume Lake Ontario fish. Despite these advisories, a survey of 655 pregnant women in the Oswego area, conducted by the authors prior to the start of this study, indicated that 8.2% of the pregnant women reported eating large quantities of fish (over 26 lbs in the past 6 years), with a total of 46% saying that they had eaten at least some fish from Lake Ontario. Since there exists a ban on commercial fishing, sport fishing accounts for all fish eaten from Lake Ontario.

With very little known about the behavioral effects in humans of the combination of chemicals found in Lake Ontario fish, and with our knowledge that women of childbearing age were being exposed to these chemicals through consumption of sport fish, the researchers of the Oswego Newborn and Infant Development Project began a prospective longitudinal study, based on babies born between 1991 and 1994, whose mothers had or had not eaten

Lake Ontario fish. Fish consumption, analytical results, and a large number of behaviors measured at birth, 3, 6, 12, 24, 36, and 48 months, comprise the entire study. This paper reports Neonatal Behavioral Assessment Scale (NBAS) (Brazelton 1984) results for the sample of newborns. Tronick (1987) has argued that the NBAS is the most standardized, valid, and reliable assessment of the newborn, one that assesses a wide range of interactive behaviors and reflexes, and is the preferred tool for examining the effects of environmental agents on the newborn. This is especially true, Tronick notes, given that behavioral environmental teratogens tend to have nonspecific subtle effects on many areas of newborn functioning.

This study represents the first large scale replication and extension of the only previous study designed to assess in humans the neurobehavioral effects of consuming environmentally contaminated fish. The Jacobsons and their colleagues (Jacobson *et al.* 1984) found associations between amounts of Lake Michigan fish consumed by mothers and behavioral changes on the NBAS in newborns. Infants born to mothers consuming the greatest amounts of contaminated fish showed: 1) more abnormally weak reflexes; 2) greater motor immaturity and more startles; and 3) less responsiveness to stimulation. Additionally, Rogan *et al.* (1986), employing a maternal body burden estimate of prenatal PCB exposure, found more highly exposed infants to have more abnormal reflexes and to be less responsive to stimulation on the NBAS, patterns similar to the Jacobson *et al.* (1984) results. The present study was designed to determine whether similar results would occur in babies born between 1991 and 1994 to mothers who had consumed Lake Ontario fish.

METHODS

Participants

Pregnant women were recruited during the physician's required 20 week sonogram visit to the office of the county's single obstetric practice. These women were initially interviewed between June, 1991, and June, 1994. The sample represents low to middle socioeconomic status (SES), and almost all of the babies are Caucasian. While a total of 1,337 out of 2,587 pregnant women volunteered (52%) to be interviewed, only 602 women represent the pool of participants for this study (only a random subset of mothers who had not eaten Lake Ontario fish were included in the control group to be described later, n = 584 were not included). By the time of hospital delivery, attrition had reduced this number to 559. An analysis of dropouts indicated that a vast majority of the lost participants were due to name changes not reported before the birth of the baby, participants who had moved from the area or who reported that their schedules were too busy to continue. Few babies in the sample exhibited risk symptoms at birth (e.g., low Apgar scores (Apgar 1953), low birthweight, premature birth, etc.), and those who did were distributed evenly across the high, low, and no fish groups.

All pregnant women over 18 years of age and seeking prenatal care were given, during their first prenatal visit, a brief description of the study by the nurse practitioner and a brochure providing additional information. Two minors (17 years of age) were also included in the sample, and parental consent was obtained in both instances. At the 20-week sonogram visit, volunteers were interviewed by a member of the study Interview Team as to Lake Ontario fish consumption, demographic and health information, and a large number of potential confounding variables (e.g., other toxic exposure). Informed consent was also obtained at this time. Fish consumption interviews included data on species of fish eaten. A chart of photographs was shown to aid identification. For each species, number of meals eaten, serving size (plastic samples of fish portions together with a baked potato and corn cob were provided on a plate), and method of preparation were obtained, for the time period prior to and during pregnancy. Contaminant values vary as a function of species of fish eaten, portion size, and method preparation, so data on these variables were collected during the interviews. Because it would be improper to equate a one pound salmon meal with a one pound perch meal, relative PCB-equivalent weights were determined according to the following algorithm:

$$\text{Exposure} = \Sigma\ \text{species}\ [\Sigma\ \text{years} \times \#\ \text{meals/yr} \times \text{serving size} \times \text{PCB load} \times \text{preparation method}]$$

species	= fish species eaten
years	= number of years Lake Ontario fish eaten from early childhood to index pregnancy
# meals/yr	= estimated number of meals consumed each year
serving size	= usual serving size (i.e., .25, .50, or 1.00 lbs)

PCB load = NYS Department of Environmental Conservation levels

preparation method = calculated by considering trimming belly fat, skin removal, whole fish (chowder) consumed.

For example, for a species given a value of 1.0 if it was trimmed and the belly fat was removed, contaminant loading was increased by .33 if the belly fat was never trimmed, and by .33 if the skin was never removed, and by .25, .15, .05, and 0.0, respectively, if it was trimmed or belly fat removed rarely, sometimes, usually, or always, respectively.

Using this algorithm, a one pound salmon or trout meal, cooked with the belly fat and skin, was given a value of 1.66, and a one pound yellow perch meal filleted with belly fat and skin removed was given a value of 0.1. Group assignments were made based on PCB-equivalent values derived by this method. PCBs were used as an indicator of toxic exposure due to fish consumption, because the largest database the New York State Department of Environmental Conservation was able to provide us with was for PCBs. Three groups were formed: high, low and a no fish eating control. Women assigned to the high fish group reported having eaten at least 40 PCB-equivalent pounds in their lifetime (n = 152). This cutoff was suggested, prior to data collection, by J. Jacobson (personal communication). Women assigned to the low fish group reported having eaten less than 40 PCB-equivalent pounds in their lifetime (n = 243). To have relatively equal numbers of women in the no fish control and high fish eating groups, the following strategy for control group assignment was established. From the pool of women reporting no Lake Ontario fish consumption, women were randomly selected in numbers equal to the high fish group. This selection and assignment process occurred monthly such that selected control group women had expected delivery dates in the same month as their high fish group counterparts (control group n = 164). At birth all participants were reinterviewed updating information obtained at the sonogram and covering the last portion of the pregnancy.

Subject characteristics are presented by group membership in Table 1. In comparing groups on characteristic variables, interval data were analyzed with ANOVA and post hoc Scheffé comparisons, and categorical data were analyzed with X^2 tests of independence.

As expected, Table 1, reveals PCB-equivalent body burden from Lake Ontario fish consumption, as estimated by a retrospective food frequency dietary assessment, differed among groups. Results of Scheffé tests (see Table 1) showed that Lake Ontario fish consumption was significantly higher in the high fish group than the low and no fish groups, which did not differ significantly. Women in the high fish group report eating on average 388 PCB-equivalent pounds of Lake Ontario fish, which is a mean of 2.3 salmon or trout meals per month (belly fat trimmed and skin removed), for a mean of 16 years. One other difference is revealed in comparing these groups. The pre-pregnancy weight for the high fish group was significantly greater (on average 10.6 lbs more) than the no fish control group (no other groups differed). While statistically significant and controlled for in the analysis, it is not clinically significant. All three groups were judged to be slightly overweight as determined by New York State Department of Health guidelines. The fact that there were no differences revealed either in weight gain during pregnancy or in nutritional variables across the sample mitigates against the clinical significance of this statistical finding.

Procedure

The Neonatal Behavioral Assessment Scale (NBAS) was administered 12-24 hrs (Time 1) and 25-48 hrs (Time 2) after birth by a member of the study's Behavioral Assessment Team who was *blind* to the babies' group assignment. They were tested in a separate quiet room, with controlled lighting and temperature. Sick babies (e.g., requiring incubation) were tested as soon as possible. The six behavioral and one reflex cluster scores defined by Lester *et al.* (1982) were employed as the preferred model of data reduction for the 28 behavioral and 20 reflex items on the NBAS. These seven clusters are: 1) Reflex, which reflects the number of abnormal elicited reflexes; 2) Range of State, which includes items relating to level of arousal; 3) Autonomic, which includes items assessing physiologic responses to stress; 4) Motor, which measures the quality of muscle tone and movement; 5) Orientation, which includes attention to visual and auditory stimuli during alert states; 6) Regulation of State, which reflects the quality of the infants' responses when aroused and ability to control arousal in response to environmental stimulation; and 7) Habitu-

TABLE 1. Background characteristics of total sample for high fish, low fish, and no fish control groups.

	High Fish (N = 152)			Low Fish (N = 243)			No Fish Control (N = 164)			
Measure	Mean	(SD)	%[a]	Mean	(SD)	%	Mean	(SD)	%	sig/ns
Demographic										
Child's sex (% male)			51.7			43.4			48.4	ns
SES[b] score	52.94	13.2		50.51	14.3		51.53	14.9		ns
Maternal education (yrs)	12.46	2.0		12.56	2.2		12.40	2.3		ns
Marital Status (% married)			59.9			65.4			59.8	ns
Maternal age	28.82	4.9		28.94	5.5		28.55	5.5		ns
Parity of child	1.18	1.1		1.13	1.1		1.18	1.3		ns
Health/Nutrition										
Pre-preg. weight (lbs)	153.01	40.6		145.15	36.8		142.41	36.5		sig[1]
Wt. gain during preg.	30.35	15.9		33.12	14.0		32.87	12.6		ns
% poor nutrition[c]			48.3			59.3			54.4	ns
Stress prior to preg.[d]	4.75	1.6		4.50	1.6		4.60	1.6		ns
Stress since preg.[d]	4.64	1.6		4.26	1.6		4.43	1.8		ns
Obstetric optimality[e]	32.99	3.3		33.58	3.4		33.30	3.2		ns
PCB-equivalent lbs[f]	388.47	859.0		10.14	17.8		00.00	00.0		
Maternal Substance Abuse[g]										
Alcohol										
Beer	10.51	46.5		7.69	28.7		4.32	16.4		ns
Wine	2.57	11.4		2.02	8.4		9.22	99.6		ns
Liquor	1.91	5.9		1.71	7.6		10.66	100.9		ns
Smoking (cig./day)	6.58	9.0		5.53	8.2		6.42	8.8		ns
Infant Birth Characteristics										
Birthweight (gm)	3480.80	511.0		3432.53	545.4		3376.91	533.2		ns
Head circumference (cm.)	34.81	4.5		34.39	1.0		34.24	1.4		ns
Gestational age (wks)[h]	39.79	1.6		39.70	1.3		39.67	1.5		ns
Ballard: Neuromuscular[i]	18.41	3.2		18.29	4.6		18.55	4.0		ns
Ballard: Physical[i]	20.38	1.8		19.94	2.0		20.08	2.0		ns

[a]Means and standard deviations are given for continuous variables, % for categorical variables
[b]Hollingshead 2-factor index (1967)
[c]Nutrition categories derived from NYS Dept. of Health
[d]Stress scale: 1 = very relaxed to 7 = very stressed
[e]Littman and Parmalee (1978)
[f]From fish consumption interview and derived from following formula:

$$\text{Exposure} = \Sigma \text{ species } [\Sigma \text{ years} \times \#\text{ meals/yr} \times \text{serving size} \times \text{PCB load} \times \text{preparation method}]$$

[g]Substance abuse variables calculated in total units for entire pregnancy
[h]Based on obstetrician's estimated day of confinement
[i]Ballard *et al.* (1979)
[1]$F(2,551) = 3.33$, $p < .05$
Scheffé comparisons (pre-pregnancy weight):
high vs. no fish, $F(2,551) = 3.98$, $p < .05$
high vs. low fish, $F(2,551) = 1.69$, $p > .05$
low vs. no fish, $F(2,551) < 1$

ation or Response Decrement, which includes items assessing the infants' reactivity to stimulation from a rattle, bell, light, and mild pin prick, followed by response decrement while in a light sleep state. Due to the sleep-state dependent nature of many NBAS items, it is difficult to obtain complete data for all clusters. For example, Habituation items must be administered while the infant is in a light sleep, and Orientation can be assessed only when the infant is in a quiet alert state. Additionally, two items in the Regulation of State cluster (consolability and self quieting behavior) require the newborn to be in crying and active alert states, respectively. As a result, the NBAS was administered to 148 high fish group newborns at Time 1, and 144 high fish group newborns at Time 2; 230 low fish group newborns at Time 1, and 228 low fish group newborns at Time 2; and 157 no fish control group newborns at Time 1, and 158 no fish control group newborns at Time 2.

Reliability

Author E.L. was certified to administer the NBAS by members of the Boston Children's Hospital NBAS training staff. He in turn trained three research scientists hired as part of the behavioral assessment team. Interobserver agreement was calculated prior to the beginning of data collection, and was defined in terms of agreements/(agreements + disagreements).

Reliabilities were calculated both allowing for the 1-point discrepancy permitted in NBAS training, and not allowing for said discrepancy. Allowing for the 1-point discrepancy yielded an interobserver agreement of 98%. Disallowing such discrepancies yielded an interobserver agreement of 92%. Six month and 1 year follow-up reliabilities were also conducted. Permitting the 1-point discrepancy yielded interobserver agreements of 95% and 98% for the 6 month and 1 year assessments respectively. Disallowing such discrepancies yielded 6 month and 1 year reliabilities of 90% and 88% respectively.

Intra-subject reliability for fish consumption was calculated for those women in the high and low fish groups who gave birth to two babies included in the study population (N = 20 pairs), and were thus given the life-time fish-consumption interview twice. While interview data regarding fish consumption generated interval data and continuous measurement, data considered for this reliability component were rank ordered. To argue that in order to be regarded as reliable over time that women be required to report *exactly* the same amount of fish consumption before the birth of both babies appears overly restrictive. Rather, it is much more realistic to argue that women's relative ordering of fish consumption at both interviews should remain relatively consistent. Therefore, a Spearman rank order correlation was calculated on the ranks of lifetime fish consumption at both interviews for the 20 pairs of data points. The resulting value, r_s = .88, reveals substantial agreement in fish consumption estimated at the two interviews.

RESULTS AND DISCUSSION

Representativeness of Sample

All women volunteering to participate in the Oswego Newborn and Infant Development Project were compared to non-volunteers served by the same obstetric practice and delivering in the same hospital. Establishing equivalence between the population of all women delivering babies in the county and the subset of women who are involved in the study permits generalization of the results to that population. Therefore, differences in demographic, labor and delivery variables were analyzed with independent sample t-tests or X^2 tests of independence. Table 2 presents these results.

Data for 780 project participants were compared to the 2,037 nonparticipants. Several statistically significant differences emerged. Both the 1- and 5-minute Apgar scores were found to be significantly different. However, when examining the magnitude of the differences in the mean scores, it is shown that each of the Apgar group scores differ by less than .15 (i.e., 8.42 versus 8.28, and 9.20 versus 9.08). Further, since the scale ranges from 0 to 10 with any score exceeding 7 considered normal, the mean values at 1 and 5 minutes clearly indicate near optimal functioning for both groups. Similarly, for the labor induction variable, associations were revealed between the variable categories and group membership. However, examination of Phi coefficients (Phi = .04) reveal there is very little strength of association for this variable. With respect to the delivery type variable statistical significance was also revealed. Inspection of the Phi coefficient (Phi = .05), however, once again reveals that although statistical significance emerged, clinical significance did not.

Considering the results reported in Table 2, we are confident that these two groups do not meaningfully differ from each other on crucial demographic.

TABLE 2. Demographic, labor, and delivery scores for study sample and hospital population.

	Study Sample[1] (n = 780) Mean(SD)	Hospital Population[2] (n = 2,037) Mean(SD)	sig/ns
Age	25.39 (5.19)	24.99 (5.56)	ns
Parity	1.19 (1.21)	1.10 (1.21)	ns
Gravida	2.59 (1.49)	2.48 (1.49)	ns
Apgar-1 min	8.42 (.81)	8.28 (1.20)	sig**
Apgar-5 min	9.20 (.56)	9.08 (0.94)	sig**
Marital Status			
% single	33.4%	35.8%	
% married	60.1%	58.3%	
% other	6.5%	5.9%	ns
Labor Induction			
% yes	8.1%	10.9%	
% no	91.9%	89.1%	sig*
Complications			
% yes	27.6%	28.9%	
% no	72.4%	71.1%	ns
Meconium Staining			
% yes	14.7%	15.9%	
% no	85.3%	84.1%	ns
Delivery Type			
% vaginal	78.7%	74.2%	
% cesarean	21.3%	25.8%	sig*
Delivery Complications			
% yes	41.0%	42.8%	
% no	59.0%	57.2%	ns

* $p < .05$
** $p < .01$
[1]The study sample subjects include extra no-fish eaters for the first 2 years.
[2]The hospital population was larger than the number of subjects we had access to at the 20 week sonogram.

labor or delivery variables. Therefore, the results may be generalized to the entire population of women delivering babies in this county.

Treatment of Confounding Variables

In considering potential confounding variables, a model was conceptualized where only those variables that represented influences temporally prior to the birth of the child were considered as confounds for physical birth measures and neonatal behavioral testing. Potential confounding variables were submitted to principal components analyses. Component scores were then used to extract variance in subsequent analyses.

In total 58 variables were constructed from responses to 159 separate items and were submitted to principal components analyses. The goal was to reduce this set of variables to a smaller number of components which would be linear functions of the original variables. The components then, and not the original variables were used to evaluate potential confounds in subsequent analyses. This strategy allows, in fact encourages, the combinations of variables to be accounted for, and is considered to be a rigorous, conservative method for the treatment of a large number of potentially confounding variables.

Data for all subjects were used to construct the component scores. Both eigenvalues (Kaiser 1960) and scree plots (Cattell 1966, Hakstian *et al.* 1982) were used as guides in determining final component structure. Standard varimax rotations were employed.

A strategy was devised where three separate components analyses were conducted. Decision rules were constructed to include like variables in each analysis. In general, the variables eligible for inclusion in the initial set of components were limited to demographic concerns (e.g., age, height, weight, SES, education, nutrition, stress, etc.). The second components analysis focused on substances consumed during pregnancy (e.g., caffeine, alcohol, tobacco products, medications, etc.), chronic medical conditions (e.g. asthma, heart disease, diabetes, rashes, etc.), and other toxic exposure (e.g., pesticides, herbicides, dry cleaning solvent, etc.). The final components analysis concentrated on labor/delivery complications and birth characteristics.

In all, 20 variables were submitted to the demographic components analysis. Eight components with eigenvalues greater than 1.00 were extracted and accounted for over 62% of the total variance. Table 3 shows the rotated matrix with only loadings greater than .30 included. The final column reveals the total percent of item variance accounted for by the extracted components.

For the second, or substance components analysis, 28 variables were submitted as possible candidates. Prior to the analysis, total units were constructed reflecting total consumption of the following substances during the entire pregnancy: caffeinated coffee, decaffeinated coffee, tea, herbal tea, beer,

TABLE 3. Varimax rotated component loadings from a principal components analysis based on 20 demographic variables.

Variable	Components 1	2	3	4	5	6	7	8	% of Variance
SES	-.88								.80
Maternal Education	.83								.70
Income	.75								.61
Marital Status	-.38	-.33							.39
Maternal Age	.32	.80							.76
Paternal Age		.74							.65
Parity		.65						-.32	.69
Miscarriages		.45			.33	.44	-.31		.68
Stress[1]			.78						.62
Stress[2]			.70						.52
Stress[3]			.69						.50
Paternal Height				.85					.75
Paternal Weight				.80					.70
Weight Gain Pregnancy					.73				.61
Pre-Pregnancy Weight					-.73	.40			.70
Maternal Height						.82			.70
Stillbirths							.77		.64
Prenatal Nutrition						-.42	-.43		.46
Race							.35		.16
History of Difficult Pregnancies								.91	.84

[1]Stress Since Learning of Pregnancy
[2]Stress Prior Year
[3]Stress between Sonogram and Delivery

wine, liquor, vitamins, prescription and over the counter medications, caffeinated soda, diet soda, and artificial sweeteners. A "total other toxic exposure" variable was created by summing responses to a series of 35 dichotomous variables. Similarly, a "total chronic medical condition" variable was generated by summing responses to 41 medical conditions. Twelve components, accounting for 63% of the total variance were extracted. Table 4 reveals the loadings (> .30) of the rotated matrix. The final column of the table represents the amount of item variance accounted for by the retained components.

The third set of component analyses focused on delivery/labor complications as well as birth characteristics. For this analysis, the OCS variable reflects the score obtained from the Obstetrics Complications Scale (Parmalee 1974, Littman and Parmalee 1978). The neuromuscular and physical variables reflect subscale scores of the Ballard Examination for Fetal Maturity (Ballard *et al.* 1979).

TABLE 4. Varimax rotated component loadings from a principal components analysis based on 28 substance variables.

	Components												
Variable	1	2	3	4	5	6	7	8	9	10	11	12	% of Variance
Cigarette[1]	.88												.79
Cigarette[2]	.86												.78
Cigarette[3]	.78												.71
Caffeine-Coffee	.69												.54
Health History		.72											.53
Drug Use		.58											.54
Prescription Drugs		.56											.55
Toxic Exposure		.42							.31				.57
Over Counter Drugs		.42					.35				.31		.60
2nd Hand Smoke[4]			.82										.74
2nd Hand Smoke[5]			.80										.74
Artificial Sweeteners				.76									.64
Diet Soda				.67									.53
Wine					.83								.71
Liquor				.38	.71								.68
Water-filtered						.68					.31		.65
Water-source						.63							.57
Plumbing						−.56					.37		.53
Years in Oswego							.72						.63
Years near Grt Lakes							−.46						.60
Decaffeinated Coffee								.71					.57
Caffeine Soda	.31							−.46					.55
Caffeine Tea									.75				.63
Herbal Tea									.44				.50
Beer										.70			.57
Decaffeinated Soda								.36					.67
Vitamins											.79		.65
Paternal Drug Use												.82	.69

[1]Cigarette smoking since learning of pregnancy
[2]Cigarette smoking between conception and learning of pregnancy
[3]Cigarette smoking between delivery and sonogram
[4]Second hand smoke between sonogram and delivery
[5]Second hand smoke prior to sonogram

In all, four components with eigenvalues greater than 1.00 were extracted accounting for 66% of the total variance in the system. Table 5 reveals loadings greater than .30 for the extracted components. The final column represents the total item variance accounted for by extracted components.

A 22 × 21 intercorrelation matrix of all component scores was generated. By chance alone, with 462 correlations each evaluated at $p \leq .05$, one would have expected to find 23 significant correlations. A total of 37 correlations were significant. Since only five correlations were greater than .20 (representing less than 5% shared variance), the orthogonality of the components is convincingly demonstrated and we can conclude that the variables are statistically independent.

As shown in Tables 3-5, very few variables evidenced complex structure. With respect to percent of variance accounted for in each item by component loadings, only five items had less than 50% variance accounted for, while the vast majority of items had greater than 60% variance accounted for. Again, the utility of the component strategy is underscored.

TABLE 5. Varimax rotated component loadings from a principal components analysis based on 10 delivery/labor variables.

Variable	Components 1	2	3	4	% of Variance
Physical	.97				.94
Neuromuscular	.96				.93
OCS	.92				.85
Weight		.79			.65
Head Circumference		.77			.60
Resuscitation			.78		.63
Meconium	.32		.60		.48
Gestational Age				-.75	.64
Anesthesia			.52	.52	.57
Sex				.48	.32

Neonatal Behavioral Assessment Scale Findings

Table 6 presents Time 1 and Time 2 means and standard deviations for the NBAS Lester clusters by group for all babies tested (Lester *et al.* 1982, Lester 1984). Analyses, however, were performed on change scores. From its inception, the NBAS was designed to reflect the changing nature of the neonate's physical, physiological, and behavioral systems (Brazelton 1990, Lester 1984). As such, it has never been deemed appropriate that a single NBAS examination score be used in data analysis. As an instrument designed to measure the dynamic aspects of newborn coping and behavior, scores representing change from day to day resulting from multiple NBAS administrations more accurately reflect the purpose of the NBAS in a research context. Further, NBAS scores reflecting change in infant and newborn behavior appear to be more predictive of cognitive, motor, and personality outcomes (Brazelton 1990). With this in mind, change scores were used in the present analyses rather than individual 12-25 hr (Time 1) and 25-48 hr (Time 2) scores.

A multivariate analysis of covariance (MANCOVA) was performed on change scores (i.e., Time 2 - Time 1) for all NBAS clusters excepting the Habituation cluster (which was treated separately due to a smaller sample size). The single independent variable was Group membership (i.e., high, low or no fish) and all derived component scores were treated as covariates. A total of 416 cases were included in this analysis (111 high fish, 181 low fish and 124 no fish). Since both Time 1 and Time 2 scores are required to calculate change scores, the change score sample sizes differ somewhat from either the Time 1 or Time 2 sample sizes. Table 7 presents unadjusted and adjusted NBAS cluster change score means for each group. Apparent differences between adjusted and unadjusted means are not great. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicolinearity were satisfactory.

Under Wilks' criterion, the combined dependent variables were significantly related to the combined set of covariates, approximate F (144,2252) = 1.22, p = .046, and to the Group variable, F (12,768) = 3.90, p < .001. The association between the depen-

TABLE 6. Groups means and standard deviations (in parentheses) for NBAS 12–24 hr (T1) and 25–48 hr (T2) cluster scores for all babies tested.

NBAS Cluste	High Fish T1	High Fish T2	Low Fish T1	Low Fish T2	No Fish Control T1	No Fish Control T2
# Abnormal Reflexes	4.09(2.39)	3.34(2.20)	3.50(2.22)	2.10(1.85)	3.62(1.92)	1.92(1.77)
Orientation	6.02(1.62)	6.30(1.30)	5.74(1.74)	6.53(1.24)	6.00(1.70)	6.86(1.28)
Motor	4.88(.98)	5.18(.96)	4.98(.81)	5.44(.74)	5.08(.88)	5.52(.70)
Range of State	3.86(.65)	3.85(.66)	3.78(.69)	3.83(.69)	3.75(.78)	3.84(.70)
Regulation of State	6.66(1.32)	6.30(1.33)	5.84(1.21)	6.28(1.11)	5.98(1.27)	6.32(1.24)
Autonomic	5.90(1.27)	5.49(1.19)	6.14(1.19)	6.26(1.12)	5.96(1.36)	6.32(1.18)
Habituation	7.41(1.02)	6.42(1.34)	7.26(1.17)	7.43(.94)	7.44(.95)	8.03(.92)

Note: For number of abnormal reflexes higher scores reflect poorer performance. For all other clusters higher scores reflect better performance.

TABLE 7. Adjusted and unadjusted change score means per group for NBAS 25–48 hr (Time 2) minus 12–24 hr (Time 1) cluster scores.

NBAS Cluster	High Fish		Low Fish		No Fish Control	
	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted
Range of State	-.05	-.04	.02	.04	.10	.06
Regulation of State	.24	.27	.46	.46	.53	.51
Motor	.32	.33	.48	.77	.46	.46
Orientation	.31	.34	1.04	.95	.93	.99
Autonomic	-.54	-.44	.16	.11	.35	.30
Reflex	-.31	-.45	-1.57	-1.45	-1.78	-1.77
Habituation	-1.10	-1.08	.18	.15	.80	.82

Note: Only those subjects without missing data (components, clusters, and Time 1 and Time 2 scores, i.e., part of the MANCOVA analysis) are included.

dent variables and covariates represents a moderate association, $eta^2 = .35$. The association is somewhat lower between the dependent variables and the Group variable, $eta^2 = .12$.

To investigate more fully the power of the covariates to adjust dependent variables, multiple regressions were run for each dependent variable (i.e., NBAS cluster) in turn, with covariates acting as multiple predictors. Substance component 12 provided adjustment to the Range of State change scores, $t(389) = 2.65$, $p < .001$. Two covariates provided adjustment to the Regulation of State scores; Demographic component 4, $t(389) = 2.09$, $p < .05$, and Substance component 8, $t(389) = 2.55$, $p < .05$. Substance component 9, $t(389) = 2.50$, $p < .05$, and Birth component 2, $t(389) = 2.88$, $p < .01$, provided significant adjustment to the Motor scores. With respect to the Orientation cluster, Demographic component 6 served to adjust the scores, $t(389) = 2.044$, $p < .05$. A single covariate, Birth component 3 adjusted the Autonomic change scores, $t(389) = 2.073$, $p < .05$. Substance component 9 adjusted the Reflex cluster change scores, $t(389) = 2.16$, $p < .05$. (See Tables 3-5 for description of component structures.)

Effects of the Group variable on the dependent variables were investigated in both univariate and stepdown analyses (shown in Table 8). The stepdown analysis was necessary owing to the significant Bartlett test of sphericity, $p < .001$. While priority levels are generally dictated by theory for a stepdown analysis, in this case theory would argue that since the NBAS is a wide-ranging test of newborn behavior, assignment of priority levels to any particular cluster would be inappropriate. It was decided to attempt to replicate the earlier Jacobson work in the most conservative manner possible. When examining the relationship between contaminated fish consumption and neonatal behavior through regression analysis, the Jacobson work reported significant F values (i.e., $p < .05$) for the Autonomic and Reflex clusters in that order. Therefore, the Autonomic and Reflex clusters were entered last in the stepdown analysis. The ordering of the four remaining non-significant Jacobson clusters was randomly determined, given the absence of compelling theoretical or empirical reasons. In this way, the Autonomic and Reflex clusters were adjusted, not only for the covariates, but for the four remaining NBAS clusters as well.

Table 8 reveals a fairly consistent pattern of findings across both the univariate and stepdown results, excepting the Orientation cluster. Both the Reflex and Autonomic clusters were found to be significant (i.e., $p < .001$). Orientation was significant in the univariate analysis (i.e., $p < .01$) but failed to reach significance in the stepdown analysis where the error rate was Bonferroni adjusted (.05 / 6 = .008).

The pattern of results can be seen in Figure 1. The left panel shows the adjusted mean change scores for the number of Abnormal Reflexes. Babies in the high fish group clearly had a smaller decrease (negative change score) in the number of Abnormal Reflexes from Time 1 to Time 2 than the low and no fish groups. The center panel shows the adjusted mean change scores for the Autonomic cluster. The high fish group was the only one to show a decrease in the autonomic score from Time 1 to Time 2. A negative change score represents a worsening in performance between the first and second testing.

TABLE 8. Univariate and stepdown tests of covariates and groups.

Effect	NBAS Cluster	Univariate df	Univariate F	Stepdown df	Stepdown F	[1]
Covariates	Range of State	24/389	1.64	24/389	1.64	.008
	Regulation of State	24/389	1.05	24/388	.98	.008
	Motor	24/389	1.56	24/387	1.62	.008
	Orientation	24/389	1.30	24/386	1.35	.008
	Autonomic	24/389	.86	24/385	.86	.008
	# Abn. Reflexes	24/389	1.04	24/384	.85	.008
Groups	Range of State	2/389	.65	2/389	.65	.008
	Regulation of State	2/389	1.07	2/388	.90	.008
	Motor	2/389	.88	2/387	.43	.008
	Orientation	2/389	4.83**	2/386	3.79	.008
	Autonomic	2/389	11.91***	2/385	10.25***	.008
	# Abn. Reflexes	2/389	11.05***	2/384	7.17***	.008

[1] Bonferroni adjustment value of alpha to keep overall error rate below .05.
** $p < .01$
*** $p < .001$

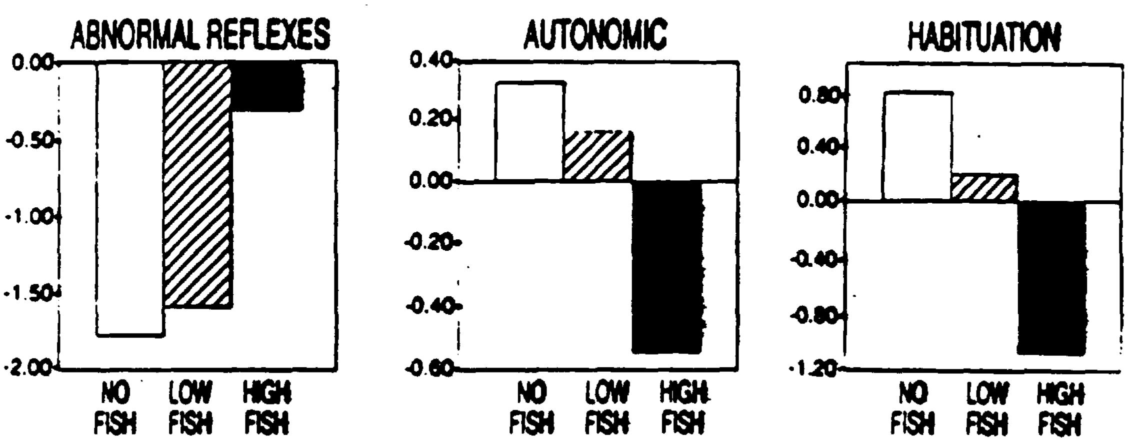

FIG. 1. Adjusted mean change scores for the no, low, and high fish groups for the Reflex (left panel), Autonomic (center panel), and Habituation (right panel) clusters. Larger negative change scores on the Reflex cluster indicate a larger decrease in the number of abnormal reflexes between Time 1 and Time 2 of testing. Positive change scores on the Autonomic and Habituation clusters indicate an improvement, negative change scores indicate a decline. Only those subjects without missing data (components, clusters, and Time 1 and Time 2 scores, i.e., part of the MANCOVA analysis) are included.

To investigate more completely the differences between adjusted group mean change scores on the Reflex and Autonomic clusters, pairwise post-hoc comparisons were conducted. The Bryant-Paulson (BP) simultaneous test procedure (Bryant and Paulson 1976), a generalization of the Tukey test, was performed. For the Reflex cluster, significant differences emerged between the high and low fish groups, BP(389) = 6.63, $p < .05$, and between the high and no fish groups, BP(389) = 6.68, $p < .05$. The comparison between the low and no fish groups did not reach significance, BP(389) = 1.10, $p > .05$. A similar pattern prevailed when comparing the adjusted mean change scores for the Autonomic cluster. Significant differences were found when comparing the high and low fish groups, BP(389)= 5.92, $p < .05$, and between the high and no fish groups, BP(389) = 8.09, $p < .05$. No differences emerged between the low and no fish groups, BP(389) = 1.64, $p > .05$.

Scheffé tests comparing each adjusted change score with 0 were done to determine if there were significant changes between Time 1 and Time 2 scores. For the Reflex cluster there was a nonsignificant change for the high fish group, $F(2,413) < 1$, but significant changes for the low and no fish groups, $F(2,413) = 49.40$, $p < .001$, and $F(2,413) = 22.43$, $p < .001$, respectively. For the Autonomic cluster there was a significant change for the high fish group, $F(2,413) = 5.80$, $p < .01$, but nonsignificant changes for the low and no fish groups, $F(2,413) = 1.88$, $p > .05$, and $F(2,413) = 2.60$, $p > .05$, respectively.

Change scores for the Habituation cluster were analyzed in a separate analysis due to missing data. Only 274 subjects had complete habituation data both at Time 1 and Time 2 administrations, and both scores are required to calculate change scores (i.e., Time 2 - Time 1). Missing data are not unusual for this cluster since it requires the newborn to be in a State 2 (light sleep) throughout the administration.

Figure 1 (right panel) shows the adjusted mean change scores for the Habituation cluster. Only the high fish group had a negative change score, which indicates a worsening in performance from the first to the second testing. The no-fish group had a positive change score, indicating an improvement in performance. The low-fish group showed essentially no change.

Group differences (i.e., high, low and no fish) for change scores were analyzed with an analysis of covariance where components from the previously reported principal components analysis were used as covariates. The results show that the covariates fail to explain significant variation, $F(24,247) < 1$. The Group membership variable is, however, significant, $F(2,247) = 27.26$, $p < .001$. Following the significant omnibus test, Scheffé pairwise comparisons were performed on adjusted means. The comparison between the high and low fish groups was significant, $F(2,271) = 29.29$, $p < .001$. Similarly, the comparison between the high and no fish groups was statistically significant, $F(2,271) = 60.17$, $p < .001$. The comparison between the no fish and low fish groups was also significant, $F(2,271) = 6.79$, $p < .01$.

Scheffé tests comparing each change score with 0 indicated a significant negative change for the high fish group, $F(2,271) = 20.02$, $p < .001$, a nonsignificant change for the low fish group, $F(2,271) < 1$, and a significant positive change for the no fish group, $F(2,271) = 10.67$, $p < .001$.

DISCUSSION

The Oswego Newborn and Infant Development Project is a prospective longitudinal study the purpose of which is to examine the behavioral effects in children of maternal consumption of Lake Ontario fish: fish contaminated with a wide range of persistent toxic chemicals. A significant strength of the study can be found in the areas of design and methodology. Essential equivalence between the study sample and population of women delivering babies in Oswego County was established on crucial demographic, labor, and delivery variables. Such equivalence strengthens the ability to generalize beyond the study sample to that population.

A potential threat to the integrity of all behavioral teratology research lies in sampling bias. Given that random assignment to groups in research of this kind is impossible and unethical, establishing equivalence between groups on behavioral nonteratogenic variables is crucial to the interpretation of findings. For example, it is well known that research on the effects of maternal cocaine consumption is heavily biased in that low income women, typically enrolled in drug treatment and recovery programs, constitute the "exposed" samples of most studies (Myers *et al.* 1992), and the nonexposed sample is quite different. As Hawley and Disney (1992) note, the most difficult methodological issue complicating studies of the behavioral teratogenic effects of illicit drugs is the selection of comparison groups. All too often significant differences other than prenatal exposure exist between study groups. Naturally if differences exist between these groups with respect to demography, health, nutrition, gestational age, or prenatal care, it becomes extremely difficult to determine the direct effect of prenatal exposure.

The comparisons reported in Table 1 establish the essential equivalence between the high, low and no fish control groups on key demographic, health, nutrition, substance use, and infant variables. Sampling bias does not appear to be a threat to this study. We have a good deal of confidence that differences that have emerged in NBAS performance are due to the fish consumption level.

This confidence is strengthened by the principal components strategy in treating potentially confounding variables. A reliable and independent set of component scores for potentially confounding variables was created, and these component scores were assessed and statistically controlled in analyzing NBAS scores. This strategy differs dramatically from and, we believe, is a more conservative and rigorous method for treating large numbers of potentially confounding variables than those which, in some a priori fashion, eliminate variables from consideration that do not show statistical significance between exposure groups, or outcomes, and/or fail to statistically control for the possible combinations of variables. Were we to use such an approach, only one variable (see Table 1), prepregnancy weight, reveals statistical differences among groups. In point of fact in the multivariate analysis of covariance, component scores explained a significant amount of variance.

Both Reflex and Autonomic cluster change scores were found to be significant after controlling for demographic, substance and birth factors via MANCOVA. High fish group babies demonstrated a greater number of abnormal reflexes and less mature autonomic response (e.g., more startles and tremors) at Time 2 than Time 1 than babies born to mothers from the other groups. There is also some evidence of less developed attention to visual and auditory stimuli (Orientation) in high fish group babies ($p < .02$), but this finding is mitigated by not having reached the Bonferroni adjusted alpha level of .008. Differences were not found on the Motor, Range of State, or Regulation of State clusters, with T2 scores on these latter two clusters identical between the high and no fish groups (see Table 6), and T1 scores slightly better in the high fish group.

The findings of poorer reflex functioning and greater autonomic immaturity in the high fish group babies are similar to the Jacobson *et al.* (1984) findings. In that study, contaminated fish consumption predicted both number of abnormal reflexes and autonomic maturity although it is important to note that the Jacobson *et al.* Autonomic cluster differs from the Lester defined cluster (Lester *et al.* 1982, Lester 1984) by including a motor maturity item, and excluding lability of skin color.

Separate analyses of Habituation cluster scores showed that babies born to high fisheaters had significantly poorer habituation recoveries than babies born to mothers in the other groups, and in contrast to low and control group babies the high group babies actually demonstrated poorer habituation responses at Time 2 than Time 1 (negative change score). This is to say that babies born to mothers who have consumed high amounts of Lake Ontario fish appear to be over-reactive to stimulation, as revealed by a delayed habituation or decrement of response to stimulation. This result is in contrast to developmental expectations and the performance of low fish and no fish control group babies. These effects were demonstrated even after controlling potential confounds via a covariate analysis. It is also interesting to note that Scheffé post hoc comparisons reveal a dose-response type relationship among fish consumption and habituation performance. The decrease in ability to habituate to mildly aversive stimuli by babies whose mothers had eaten high amounts of Lake Ontario fish is similar to results found in laboratory rats fed Lake Ontario salmon. These rats, and their offspring tested in adulthood, are hyper-reactive to negative events such as mild electric shocks and frustrative reductions in amount of a food reward (Daly 1992).

While significant and to a large extent confirmatory of the NBAS findings of Jacobson *et al.* (1984) and Rogan *et al.* (1986), the importance of these findings is mitigated by several unknowns at this time. First, while the NBAS has demonstrated utility in studies of high risk infants and in studies of effects of obstetric medication and effects of maternal substance abuse (Brazelton *et al.* 1987), evidence for the predictive validity for later behavior of NBAS scores in non at-risk population is limited. There is evidence that a focus on patterns of change in neonatal behavior as revealed in repeated administrations of the NBAS is predictive of later cognitive development. Both Lester (1984) and Nugent *et al.* (1984) have demonstrated relationships between NBAS change over time and 18 month and 3 year measures of intelligence. The Lake Michigan Maternal Infant Cohort Study has continued to show cognitive effects beyond their initial NBAS findings. These findings include, at 7 months of age less preference for a novel stimulus on the Fagan Test of Infant Intelligence (Jacobson *et al.* 1985), and, at 4 years of age poorer short term memory as measured by the McCarthy Scales (Jacobson *et al.* 1992). Nevertheless, future cognitive, and even social and emotional development may be differentially related to patterns of change in NBAS clusters, and we are at this time not well prepared to hypothesize what the long-term significance of the present NBAS findings might be.

Secondly, while fish consumption is the indicator variable for toxic exposure in this report, and equivalent PCB pounds were used to estimate potential toxicity among species of fish, we simply do not know what the specific nature of its behavioral teratogenic influence might be. Relationships between NBAS scores and total PCBs, PCB congener patterns, DDE, HCB, lead, and mercury in the cord blood of babies and hair of the mother in the high fish and no fish control groups await analysis. In addition, a piece of placental tissue has been tissue-banked for future analysis.

In addition to finding behavioral differences, the Jacobson study revealed two statistically significant physical differences in newborns of mothers who consumed Lake Michigan fish. Exposed babies were lighter at birth, and had a smaller head circumference (Fein *et al.* 1984). A more recent study of newborns whose mothers had consumed Lake Michigan fish found the opposite results: babies whose mothers ate Lake Michigan fish had a higher birth weight, but only if the weight gain of the mother was less than 34 lbs (Dar *et al.* 1992). The authors of this study point out that the fish consumption levels of their subjects were lower than in the Jacobson study. As no behavioral data were collected by Dar *et al.*, a comparison on this dimension was not possible. The present study found no significant physical differences, yet did find behavioral differences in newborns as measured by the NBAS. It would appear that behavioral measures may be more sensitive measures of the effects of the pollutants in Great Lakes fish than physical measures.

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